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Research Article

Microbial quality and antibiotic susceptibility profiles of bacterial isolates from borehole water used by some schools in Ijebu-Ode, Southwestern Nigeria

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Abstract: This study was carried out to investigate the microbial quality and antibiotic susceptibility profiles of bacterial isolates from borehole water used by some schools in Ijebu-Ode, Southwestern Nigeria. Borehole water samples were taken from twelve secondary schools over a period of three weeks in September, 2012 and investigated for the presence of indicator organisms such as total coliforms, faecal coliforms, enterococci, heterotrophs and enterobacteriaceae using the spread plate technique. The membrane filtration method was adopted for the isolation pathogenic bacteria. Organisms were further identified using standard methods. Antibiogram of isolates was determined using the Kirby-Bauer method. Total coliform count ranged from 11 (as in sample A) to 2.88 x 10^2 cfu/100 ml (as in sample F). Total heterotrophic count was least in sample A (49 cfu/100 ml) and too numerous to count (TNTC) in sample C. The incidence of faecal coliform was lowest in sample I (9 cfu/100 ml) and highest in sample H (1.18 x10^2 cfu/100 ml). Genera of bacteria identified were *Staphylococcus aureus, E. coli, Klebsiella* sp, *Pseudomonas aeruginosa, Enterobacter* sp, *Salmonella* sp. and *Serratia* sp. *Escherichia coli* was found to be sensitive to all antibiotics but augmentin. Highest level of resistance was exhibited by *Enterobacter* sp. It was concluded that the borehole water used by school children in Ijebu-Ode is of poor microbial quality. A possible follow up would be necessary to identify the sources of contamination and disinfection of water in storage tanks before distribution through the school taps is also recommended as a short term solution.
Keywords: Borehole, water, antibiotics, bacteria, schools, children, reservoir

INTRODUCTION

Groundwater provides potable water to an estimated 1.5 billion people worldwide daily and has proved the most reliable resource for meeting rural water demand in sub-Saharan Africa. Groundwater is usually assumed to be a very good source of potable water due to purification property of soil. However, underground water may be subjected to pollution and may not be as safe as is generally assumed. Groundwater begins with precipitation that seeps into the ground. The amount of water that seeps into the ground will vary widely from place to place, depending on the slope of the land, amount and intensity of rainfall, and type of land surface. Porous, or permeable, land containing lots of sand or gravel will allow as much as 50 percent of precipitation to seep into the ground and become groundwater [1]. In less permeable areas, as little as five percent may seep in [2]. The rest becomes runoff or evaporates. Over half of the fresh water on Earth is stored as groundwater. As water seeps through permeable ground, it continues downward until it reaches a depth where water has filled all the porous areas in the soil or rock. This is known as the saturated zone. The top of the saturated zone is called the water table.

Ijebu-Ode is a water scarce town, and groundwater from wells, private or public boreholes is the main water source in many rural areas for cooking, drinking, and other domestic activities. In some primary schools, boreholes are used to provide drinking water to school children and communities around the school when there are no other water sources available. Previous studies in some rural areas of Limpopo Province in South Africa have reported poor quality of ground water consumed by the population [3]. Samie *et al.* [2] also reported similar result in their study of borehole water used by schools in Greater Giyani Municipality, Mopani District, South Africa. However, there are very little or no published data available on microbiological properties of water resources used in public schools in Ijebu - Ode, Northwestern Nigeria where children might be at high risk of diarrheal diseases [4]. Elsewhere, outbreaks of diarrhoea have been reported in rural areas of Limpopo Province due to contaminated borehole water [1].Groundwater provides potable water to an estimated 1.5 billion people worldwide daily [5]. and has proved the most reliable resource for meeting rural water demand in sub-Saharan Africa [6].

Boreholes equipped with handpumps are a common technology adopted by poor rural communities, and there are currently approximately 250,000 handpumps in Africa [7]. In 1994, it was estimated that 40-50% of handpumps in sub-Saharan Africa were not working [8]. This is backed up by more recent data from Uganda [7] and South Africa [9], which indicate similar operational failure rates. An evaluation in Mali in 1997 found 90% of pumps inoperable just one year after installation [10]. The primary reason for these high failure rates, and hence low sustainability, is insufficient attention to operation and maintenance of the pump [11,12]. This borehole itself, however, is sometimes the source of the problem. This study aims to investigate cases in which it is the borehole, rather than the pump, that has failed.

A large proportion of the World's people do not have access to improved or microbiologically safe sources of water for drinking and other essential purposes: IDRC [13] has estimated that 1.1 billion people do not have access to "improved drinking-water sources". Consumption of unsafe water continues to be one of the major causes of the 2.2 million diarrheal disease deaths occurring annually, mostly in children [2, 13]. Despite major efforts to deliver safe, piped, community water to the World's population, the reality is that water supplies delivering safe water will not be available to all people in the near term [14, 15].

The millenium declaration established as a goal halving the proportion of the global population without access to safe water by 2015. One reason for this is that fecal contamination of source and treated water is a persistent, worldwide problem. Sanitation coverage is inadequate in many parts of the world and is likely to persist for the foreseeable future. Fecal contamination of source and treated water is further exacerbated by increasing populations, urban growth and expansion, peri-urban settlement and continued and perhaps increasing pollutant transport into ground and surface water due to deforestation, global climate change, recurrent disastrous weather events (hurricanes, cyclones, floods, tsunamis, etc.) and increasing coverage of the earth's surface with impervious materials [16]. Current estimates of the number of people using microbiologically unsafe water are probably low. This is because the assumptions about the safety or quality of water based on its source, extent of treatment or consumer handling do not take into consideration several well-documented problems. One problem is that so-called protected or improved sources, such as boreholes and treated urban supplies, can still be faecally contaminated and deliver microbially unsafe water [14]. In some cities the water systems abstract unsafe water from unprotected or contaminated sources and deliver it to consumers with no or inadequate treatment, yet these water systems are classified or categorized as improved and safe. Another problem contributing to the underestimation of the population served by unsafe water is contamination of water during distribution whether water is piped or carried into the home [16].

It is important to assess the characteristics of groundwater resources particularly in rural communities where groundwater is used on a daily basis. The population increase in this study area resulted in high demand for potable water for drinking, cooking, washing and purposes. Because of this, groundwater became the predominant source of water for domestic use and other purposes in the rural communities [11]. The microbiological quality of water used in these villages has not been studied and there are no data on the diversity and antibiotic susceptibility profiles of bacterial isolates from these villages. Children are generally more vulnerable to intestinal pathogens and it has been reported that about 1.1 million children die every year due to diarrheal diseases [2, 17, 18].

It, therefore, becomes imperative to determine the quality, microbial diversity and antibiotic susceptibility profiles of microbial isolates from water sources consumed by the school children, because they are vulnerable to different kinds of diseases since their immune systems are still developing. In Malawi 3000 children were infected with diarrhoea in 2005 and 1000 of them died [19]. The latter study reported that 43% of the population obtained water from wells, streams and other unreliable water sources leaving them prone to water related diseases including cholera. Bacteria may manifest resistance to antibacterial drugs through a variety of mechanisms. Some species of bacteria are innately resistant to one class of antimicrobial agents. In such cases, all strains of that bacterial species are likewise resistant to all the members of those antibacterial classes. Of greater concern are cases of acquired resistance, where initially susceptible populations of bacteria become resistant to an antibacterial agent and proliferate and spread under the selective pressure of use of that agent. Several mechanisms of antimicrobial resistance are readily spread to a variety of bacterial genera. The aim of this study was to assess the total quality of borehole water used in twelve schools in Ijebu-Ode, Southwestern, Nigeria and to determine the diversity and antibiotic susceptibility profiles of potential bacterial pathogen isolated from these sources.

MATERIALS AND METHODS

Study area

Ijebu Ode is a Local Government Area and city located in south-western Nigeria, close to the A121 highway with an estimated population of 222,653 (2007). The city is located 110 km by road north-east of Lagos; it is within 100 km of the Atlantic Ocean in the eastern part of Ogun State and possesses a warm tropical climate. Ijebu - Ode has 39 Public Primary Schools, 14 Public Junior Secondary school, 13 public Senior Secondary Schools, 110 approved Private Nursery and Primary Schools and 22 approved Private Secondary Schools. It is the second largest city in Ogun

State after Abeokuta. Since pre-colonial times it has been the capital of the Ijebu kingdom. The LGA has an area of 192 km² and a population of 154,032 at the 2006 census. The postal code of the area is 120. The largest city inhabited by the Ijebus, a sub-group of the Yoruba ethnic group who speak the Ijebu dialect of Yoruba; it is historically and culturally the headquarters of Ijebuland.

Identification of schools

Different schools in Ijebu-Ode were visited with a view to formulating research questions. The questions asked centered on the availability of boreholes in schools, their uses, water reservoirs such as tanks, if there was prior treatment of borehole water before use and any other related concern within the school community. For the purpose of the study, only twelve schools that have and use borehole water in Ijebu-Ode were selected for analysis using stratified random sampling technique.

Microbiological analysis of the borehole water samples

This was done by investigating the presence of indicator organisms such as total coliforms, enterococci, faecal coliform and heterotrophs, and detecting pathogenic bacteria such as *Salmonella*, *Shigella* and *Escherichia coli*.

Collection of water samples

All the sampling points were selected within the chosen schools. Samples were collected over period of three weeks in September, 2012. The borehole water sources that were selected were those that were used for drinking and for other domestic purposes such as cleaning. 500 ml glass sampling bottles were used. Sampling bottles were pre-sterilized in an autoclave for field use. A burner was used to sterilize the faucet of the borehole source and water was left to run for 4 - 6 mins before collection. Collected samples were kept at 4°C in the cooler box packed with ice and transported to the laboratory for analysis within six hours [20].

Assessment of total microbial quality

Preparation of culture media

McConkey Agar was used for the isolation of Enterobacteriaceae, m- enterococcus agar was employed for the isolation of enterococci and m-Endo agar for the determination of total coliform. Faecal coliform count was determined using Eosin Methylene Blue (EMB) agar medium employing the pour plate technique and Plate Count Agar (PCA) was used for the enumeration of heterotrophs. The media were prepared a day before going to the sampling site and in accordance with the manufacturer‘s instruction depending on the volume needed. After preparation, media were allowed to cool, and then dispersed into Petri dishes.

Membrane filtration and culture

Microbial quality assessment was done using the standard membrane filtration technique as described by Ziel *et al.* [21]. Briefly, samples (100 ml) were filtered through 47 mm microsep membrane filter paper of 0.45 µm pore size. Using sterile forceps, the membrane filters were removed from the filtration cup and transferred to the Petri dishes of defined sizes containing the appropriate media for the culture of bacteria of interest. For total coliform, the plates were incubated at 37ºC for 24 h; for faecal coliform and heterotrophs the plates were incubated at 37°C for 48 h; and for enterococci, the plates were incubated at 44.5°C for 24 h. After 24 h of incubation, number of bacterial colonies was determined using BOECO colony counter and expressed as colony forming units (CFU) per 100 ml. According to Klein and Bickmell [22] the maximum number of colonies that can accurately be counted on a plate is usually 300. Therefore, the counts for plates with over 300 colonies were regarded as numerous. Ten fold serial dilutions were made in order to obtain countable plates. 1 ml of sample was added to 9 ml of sterilized distilled water. The bacterial suspension was mixed by rotating between the hands, and 1 ml of the suspension was transferred to 9 ml of sterile distilled water labelled 10^{-2}. The same procedure for mixing was employed for 10^{-3} and 10^{-4}. One mil of the sample dilution was poured into appropriate agar plate. A spread plate technique was employed. After agar has hardened for about 5 min, the plates were inverted and incubated at 37°C for 24 h. After incubation, colonies were counted using colony counter.

Isolation and identification of selected pathogenic bacteria

The membrane filtration method as described under total microbial quality was employed. Presumptive identification of colonies was determined on the basis of cultural characteristics. Brown, green, yellow, pink and cream white colonies on Salmonella-Shigella Agar indicated *Salmonella* and *Shigella* species and pink and cream white colonies on m-Endo Agar indicated *E. coli.* Subculturing was performed in order to obtain pure colonies by streaking into fresh plates. Pure colonies were incubated at 37°C for 24 h [23,24].

Antibiogram determination

The Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the bacterial isolates. Antibiotic multidisks used consisted of Septrin (30 µg), Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxicillin (30 µg), Augmentin (30 µg), Gentamicin (10 µg), Perfloxacin (30 µg), Tarivids (10 µg) and Streptomycin (30 µg).The medium used was Mueller Hinton (MH) agar. Pure cultures of organisms were enriched in nutrient broth and incubated at 37^0C to a turbidity of 0.5 Macfarland standards. The MH agar was inoculated by streaking using sterile cotton swab of each of the cultures. The antibiotic disks were applied using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30minutes to allow for diffusion of the antibiotics and the plates were incubated inverted at 37^0C for 24 hours. Results were recorded by measuring the zone of inhibition and comparing with the NCCLS interpretive performance standard for antimicrobial disk susceptibility testing [25,26].

RESULTS AND DISCUSSION

Table 1 summarized the results of the microbial quality of the sampled borehole water from twelve secondary schools in Ijebu-Ode, Nigeria. Total coliform count ranged from 11 (as in sample A) to 2.88 x 10^2 cfu/100 ml (as in sample F). The counts exceeded the 5 cfu/100 ml which is the maximum recommended limit [27]. Total heterotrophic count was also least in sample A (49 cfu/100 ml) and highest with 3.77 x 10^2 represented by TNTC in sample C. The heterotrophs counts exceeded the recommended maximum limit of 100 cfu/100 ml [27] except for borehole waters from samples A and I which had tptal heterotrophic count of 49 and 71 respectively. The incidence of faecal coliform was lowest in sample I (9 cfu/100 ml) and highest in sample H (1.18 10^2 cfu/100 ml). However, according to DWAF and WRC [28], the maximum limit for no risk of faecal coliform is 0 cfu/100 ml. Enterococci were not encountered in all samples (Table 1); this was in line with the recommended minimum limit of 0 cfu/100 ml. The distribution of borehole water quality showed that poor water quality is likely to occur in schools which use borehole water only (no surface water from the bulk supply). The cluster could have been influenced by the position of the boreholes which were close to the pit latrines.

Table 1: The total microbial quality of borehole water sources used by schools in Ijebu-Ode, Northwestern, Nigeria

Sampling point	Dates of sample collection	Total coliform	Heterotrophs	Faecal coliform	Enterococci
Limit for no risk		0-5 cfu/100 ml	0-100 cfu/100 ml	0 cfu/100 ml	0 cfu/100 ml
A - Epic International College, Irewon Road, Ijebu-Ode	10/09/2012	11	49	8	0
B - Christ Church High School, Sabo, Ijebu-Ode	10/09/2012	1.29 x 10^2	2.13 x 10^2	50	0
C - Muslim Girls' High School, Eruwon Road, Ijebu-Ode	10/09/2012	1.76 x 10^2	TNTC	1.07 x 10^2	0
D - Sanni Luba Comprehensive High School, Eruwon Road, Ijebu-Ode	10/09/2012	2.39 x 10^2	TNTC	1.02 x 10^2	0
E - Taiye Solarin University of Education Secondary School, Igbeba, Ijebu-Ode	17/09/2012	91	TNTC	64	0
F - Ansarudeen Secondary School, Ota Street, Ijebu-Ode	17/09/2012	2.88 x 10^2	2.96 x 10^2	36	0
G - Angilcan Girls' Grammar School, Obalende, Ijebu-Ode	17/09/2012	2.46 x 10^2	2.84 x 10^2	1.14 x 10^2	0
H - Ifesowapo Comprehensive High School, Imodi-Imosan, Ijebu-Ode	17/09/2012	2.18 x 10^2	2.22 x 10^2	1.18 x 10^2	0
I - Ijebu-Ode Grammar School, Abeokuta Road, Ijebu-Ode	24/09/2012	40	71	7	0
J - Molipa High School, Molipa Exprees Road, Ijebu – Ode	24/09/2012	1.40 x 10^2	2.0 x 10^2	12	0
K - Our Lady of Apostle, Epe Garage, Ijebu-Ode	24/09/2012	23	1.38 x 10^2	26	ND
L - Adetola Odutola Memorial High School, Ijebu-Ode	24/09/2012	42	2.19 x 10^2	12	0

TNTC - Too Numerous To Count

Table 2: Morphological and biochemical characteristics of bacterial isolates from borehole water samples in Ijebu-Ode, Southwestern Nigeria

Parameters	Most Probable Isolates							
	Staphylococcus aureus	***E. coli***	***Klebsiella* sp**	***Pseudomonas aeruginosa***	***Enterobacter* sp**	***Salmonella* sp**	***Proteus* sp**	***Serratia* sp**
Gram's reaction	+	-	+	-	+	-	-	-
Catalase test	+	+	+	+	-	+	-	-
Citrate test	-	+	+	+	+	+	-	+
Oxidase test	-	-	+	+	-	-	-	-
Coagulase test	+	-	-	-	-	-	-	-
Indole test	-	+	-	-	-	+	+	-
Urease activity	+	-	+	NA	NA	-	+	-
Cellular morphology	Cocci	straight rods	rods	Rods	Cocci	rods	rods	Rods
Growth on blood agar (colony)	creamy white	circular	large white	greenish	Creamy	NA	NA	NA
Growth on Mannitol salt agar	bright yellow	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Growth in MacConkey agar	N/A	red/ pink	mucoid	pale	Pink	pale	pale	pink/red
Glucose	A	A/G	A/G	N/A	A/G	A/G	A/G	A/G
Lactose	A	A/G	A/G	N/A	A/G	-	-	-
Sucrose	A	A	A	N/A	A	-	-	-
Mannitol	A	A	D	N/A	A	A/G	A/G	-
Maltose	A	A	N/A	N/A	A	A/ G	-	-

- (No growth), + (growth), N/A (Not applicable), A (Acid), A/G (Acid and Gas)

Eight genera of bacteria were isolated from the sampled borehole waters. These were identified as *Staphylococcus aureus, E. coli, Klebsiella* sp, *Pseudomonas aeruginosa, Enterobacter* sp, *Salmonella* sp. and *Serratia* sp. (Table 2).

In this study, members of the *Enterobacter* were identified in high numbers as compared to other organisms. It was found to be present in all borehole water samples in varying degree (Table 3). The identification of this kind of bacteria in water justifies the contamination of water by faecal material from warm blooded animals (humans). This further justifies the fact that one of the potential sources of borehole water pollution is sewerage.

Table 3: Bacteria detected from the borehole water sources from some schools in Ijebu-Ode, Northwestern Nigeria

Sampling point	Bacterial Isolates
A - Epic International College, Irewon Road, Ijebu-Ode	*Klebsiella* sp., *Enterobacter* sp., *Proteus* sp
B - Christ Church High School, Sabo, Ijebu-Ode	*Enterobacter* sp., *Proteus* sp., *Serratia* sp
C - Muslim Girls' High School, Eruwon Road, Ijebu-Ode	*Enterobacter* sp., *Staphylococcus aureus*
D - Sanni Luba Comprehensive High School, Eruwon Road, Ijebu-Ode	*Enterobacter* sp., *Klebsiella* sp., *Salmonella* sp., *Serratia* sp., *Proteus* sp., *Staphylococcus aureus*
E - Taiye Solarin University of Education Secondary School, Igbeba, Ijebu-Ode	*Serratia* sp., *Pseudomonas aeruginosa, Enterobacter* sp., *Proteus* sp., *Staphylococcus aureus*
F - Ansarudeen Secondary School, Ota Street, Ijebu-Ode	*Serratia* sp., *Enterobacter* sp., *Pseudomonas aeruginosa*
G - Angilcan Girls' Grammar School, Obalende, Ijebu-Ode	*Klebsiella* sp., *Serratia* sp., *Enterobacter* sp., *Pseudomonas aeruginosa*

H - Ifesowapo Comprehensive High School, Imodi-Imosan, Ijebu-Ode	*Serratia* sp., *Enterobacter* sp., *Pseudomonas aeruginosa, Klebsiella* sp
I - Ijebu-Ode Grammar School, Abeokuta Road, Ijebu-Ode	*Serratia* sp., *Enterobacter* sp., *Pseudomonas aeruginosa, Klebsiella* sp.
J - Molipa High School, Molipa Exprees Road, Ijebu – Ode	*Enterobacter* sp., *Klebsiella* sp., *Serratia* sp.,
K - Our Lady of Apostle, Epe Garage, Ijebu-Ode	*Pseudomonas aeruginosa, Klebsiella* sp., *Enterobacter* sp., *Proteus* sp., *Staphylococcus aureus*
L - Adetola Odutola Memorial High School, Ijebu-Ode	*Klebsiella* sp., *Enterobacter* sp., *Proteus* sp

The occurrence of *Enterobacter* sp constituted 28.85% making it the most prevalent organism in this study. This was followed by *Klebsiella* sp and *Serratia* sp with same percentage frequency of 17.31%. *Proteus* sp and *Pseudomonas aeruginosa* had same percentage occurrence of 13.46%; followed by *Staphylococcus aureus* and *Salmonella* sp with percentage frequency of 7.69% and 1.92%, respectively (Fig 1).

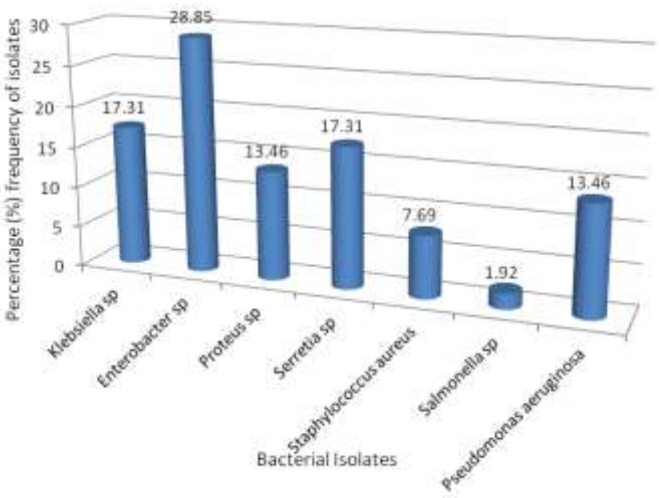

Fig 1: Percentage frequency of borehole water sources from some secondary schools in Ijebu-Ode, Nigeria

Escherichia coli was found to be sensitive to septrin, chloramphenicol, sparfloxacin, ciprofloxacin, amoxicillin, gentamicin, perfloxacin, tarivids and Streptomycin. It is, however, resistant to augmentin. Highest level of resistance was exhibited by *Enterobacter* sp as shown by its resistance to six out of ten antibiotics assayed in this study. The illustrations given in Table 3 indicated that most of the isolates were resistant while some were moderately susceptible and the remaining few sensitive

Tables 4: The antibiotic susceptibility patterns of bacterial isolates from borehole water samples used by some schools in Ijebu-Ode, Southwestern Nigeria

Antibiotic	Conc. (µg)	* Mean Diameter of Inhibition Zone (in mm)							
		E. coli	***Klebsiella* sp**	***Salmonella* sp**	***Serratia* sp**	***Enterobacter* sp**	***Proteus* sp**	***Pseudomonas* sp**	***Staphylococcus* sp**
Septrin	30	15	13	13	9	0	12	11	0
Chloramphenicol	30	16	13	14	12	0	14	12	7
Sparfloxacin	10	18	18	17	16	0	15	16	13
Ciprofloxacin	10	17	11	17	0	10	12	14	0
Amoxicillin	30	14	16	18	0	0	0	20	15
Augmentin	30	11	13	10	0	0	13	0	5
Gentamicin	10	18	10	13	13	10	8	11	0
Perfloxacin	30	15	18	14	17	16	11	18	12
Tarivids	10	19	17	17	14	13	13	18	17
Streptomycin	30	14	10	13	10	5	10	12	9

*Data showed mean results of three observations, Key <7 – Resistance, 8-13 – Moderate , >14 – Sensitive

Water meant for human consumption should be safe and acceptable and must be free from all pathogenic organisms. According to guidelines for drinking water quality, the results of the present study indicated that all the borehole water sources tested were of poor microbiological quality [27]. The present study indicated very high level of contamination of borehole water used by school children in Ijebu-Ode, Northwestern Nigeria as well as a high diversity of bacterial organisms and high levels of antibiotic resistance by the isolated organisms. This indicated that boreholes sampled in the different schools in Ijebu-Ode were very poor as shown by high counts of bacterial indicators.

Similar results have been described by other authors [3,29] on studies done in different rural communities of South Africa. However, the sources of this high contamination in the boreholes have not been investigated. Several sources of contamination could be suggested and could include the possibility of contamination from pit latrines. In fact, the construction of boreholes in these areas does not always respect the location regulations to make sure that these boreholes are not situated close to pit latrines; for example, which might be sources of contamination. Previous studies in Zimbabwe indicated that pit latrines were microbiologically impacting on groundwater quality up to 25 m lateral distance [30]. The distances of boreholes used as sources of water in most of schools under study did not obey the allowable distance of 100 m in sandy soil [27]. Other schools in the region hosting boreholes were also more or less close to pit latrines. Considering the schools far away from the pit latrines (>100 m), the impact of the sanitation is not likely to be highly pronounced except if there is high hydraulic variation in terms of groundwater level that promote high transport of pollutants towards the borehole. However, this hypothesis remains to be demonstrated. According to the Water Quality Guidelines set by DWAF [27], the results of the present study mostly exceeded the limits of microbiological quality for all the boreholes. Therefore, the water is not suitable for human consumption.

Drinking water from the boreholes can pose serious health effects to consumers; the poor microbiological quality may be due to very many closely spaced septic systems in a limited area [31]. For all the boreholes, contamination may be due to lack of sewer pipe for discharge of sanitary waste into the treatment plant. Lack of sewer lines results in underground disposal of sewage into the aquifer. This results in loss of microbiological quality of groundwater. Pit latrines located next to groundwater sources may be cause of contamination into the underground aquifer. Lack of sanitary education has resulted in poor microbiological quality of groundwater. This is because the schools just locate the pit latrines without measuring the distance between the borehole and the pit latrines. In a study in Kenya, water sources mainly wells close to pit latrines (within 15 m) were found to be contaminated with all the wells (100%) found to be containing total as well as fecal coliforms (thermo tolerant) while tap water was not contaminated, indicating the possibility of pit latrines being a major source of contamination in this case [32].

However, the authors also suggested that contamination through surface runoff during rains was also plausible, as indiscriminate excreta disposal particularly by children was also common. Nyati [33] showed that the quality of borehole water supplies in Zimbabwe showed a seasonal fluctuation, with higher coliform counts in the wet season from November to March, while municipal and mining compound water supplies were of satisfactory microbial and chemical quality. Although, all the water sources tested in the present study were contaminated, the sources of contamination though speculated remains to be confirmed.

Similar bacterial profile was described in Nigeria where *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., and *Staphylococcus aureus* were isolated from samples from boreholes [34]. In a study in Finland, shallow groundwater down to a depth of 16.2 m on average contained more biomass and cultivable microorganisms than did deep groundwater, except in a zone at a depth of approximately 300 m where the average biomass and number of cultivable microorganisms approached those of shallow groundwater [35]. The presence of such bacteria might result in diseases and poor health of the children consuming that water. *Y. pseudotuberculosis* is known to cause fever and acute abdominal pains disease outbreak in school children.

The present study has indicated low levels of antibiotic resistance among all the bacterial isolates. However, high level of resistance shown by *Enterobacter* sp, which was most prevalent in this study, is of great concern. This is in line with studies in Uganda which also described lower antibiotic resistance with less than 50% resistance to ampicillin [36]. Antibiotic resistance to gentamicin was 39 and 34% to amikacin. These resistance rates are quite high compared to those previously described among organisms isolated from river water where resistance to amikacin was less than 10% and resistance to gentamicin was less than 25% [29].

In a recent study in Poland, high resistance to erythromycin was observed among enterococci isolated from surface water reaching resistance level of 50% [37]. In another study in Alice, South Africa, *V. fluvialis* showed 100, 90, 70 and 80% resistances to trimethoprim, penicillin, cotrimoxazole and streptomycin, respectively, while 92, 82, 90 and 100%

of cephalothin resistances were exhibited by *Vibrio vulnificus, Vibrio parahaemolyticus, V. fluvialis* and *Vibrio metschnikovii* respectively [38]. Increase of antibiotic resistance has also been observed among organisms isolated from water samples in Brazil where high indices of resistance to Imipenem, Cephalothin and Ampicillin were observed [39]. High numbers of indicator and pathogenic bacteria were detected in this study.

CONCLUSION

It was concluded in this study that the borehole water used by school children in Ijebu-Ode is of poor microbial quality. It is recommended that a possible follow up would be to identify the sources of contamination and it is also recommended that storage tanks should be disinfected before distribution through the school taps as a short term solution. There is also need to carry out a comprehensive epidemiological study to determine the number of people suffering from diseases or illnesses related to the microbial water quality problems identified in the area of study. This will provide information on the actual health problems on ground as well as contribute to the use of untreated groundwater in schools. This will lead to recommendation of realistic remediation methods for each specific health problem. Information obtained would be valuable in the design and implementation of intervention strategies if required. Hence, this will enable the provision of data available to indicate that groundwater in the study areas does not meet the national guidelines of water for human consumption unless treated before use. High numbers of indicator and pathogenic bacteria were detected in this study. Considering the long term impact of diarrhea in children [40], it is imperative that actions have to be taken in order to correct the quality of water in the boreholes consumed by children. Interventions such as the implementation of point of use water treatment could be advocated as has been recommended elsewhere.

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